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# HUMIDITY AND TEMPERATURE REQUIREMENTS OF SELECTED FUNGI

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UNIVERSITY OF FLORIDA GAINESVILLE, FLORIDA

OCTOBER 1961

Willy Hickory

CONTRACT No. AF 33(616)-6387

BIOMEDICAL LABORATORY
AEROSPACE MEDICAL LABORATORY
AERONAUTICAL SYSTEMS DIVISION
AIR FORCE SYSTEMS COMMAND
UNITED STATES AIR FORCE
WRIGHT-PATTERSON AIR FORCE BASE, OHIO

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#### FOREWORD

This report was prepared by the Engineering and Industrial Experiment Station of the University of Florida, Cainesville, Florida, under contract AF 33(616)-6387. This contract was initiated under Project 7312, "Finishes and Materials Preservation," and was completed under Project 7165, "Health Hazards of Materials and Radiation," Task 71837, "BW/CW Defense." The work was administered under the direction of the Biomedical Laboratory, Aerospace Medical Laboratory, Aeronautical Systems Division, with Dr. A. E. Prince of the Biospecialties Section, Physiology Branch, Biomedical Laboratory, serving as contract monitor.

This report covers work conducted from 1 April 1959 through 31 August 1960.

#### ABSTRACT

This research was conducted to investigate the viability of spores of selected fungi at various combinations of relative humidity between 60 and 95 percent and temperatures between 50° and 100° F. The microorganisms were selected because they were known to be a cause of, or associated with, fungal deterioration of Air Force materiel. Spore germination was usually prevented when the relative humidity was below 70 percent regardless of temperature. Reducing the temperature at R.H. values below 85 percent may retard spore germination and growth slightly. Above 90 percent R.H., any reduction in temperature has very little effect on spore germination.

PUBLICATION REVIEW

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## HUMIDITY AND TEMPERATURE REQUIREMENTS OF SELECTED FUNGI

#### INTRODUCTION

These investigations were initiated to establish the humidity and temperature requirements of selected fungi which are known to be associated with the deterioration of material. An attempt was made to determine the maximum relative humidity which may be allowed and still not permit germination of spores of the microorganisms involved in deterioration. In addition to an investigation of the relation of relative humidity to spore germination, consideration also was given to temperature and time relations to obtain more complete data on the overall requirements of many fungi involved in deterioration.

The microorganisms selected for these investigations are listed below along with their American Type Culture numbers:

Name	ATCC No.
Pullularia pullulans	9349
Helminthosporium species	1680
Alternaria solani	1806
Memmoniella echinata	2373
Penicillium citrinium	805
Aspergillus glaucus	140
Cladosporium fulvum	1671
Rhisopus arrhizus	1526
Monilia brunnea	1686
Rhizopus stolonifer	10404
Aspergillus fumigatus	6285
Penicillium funiculosum	9644
Aspergillus midulans	188
Aspergillus niger	6275
Trichoderma species	9645
Myrothecium verrucaria	2003
Aspergillus tamarii	10836
Chaetomium globosum	6205
Penicillium ochrochloron	9112
Botrytis cinerea	1648
Curvularia lumata	2380

It will be noted that Phycomycetes, Ascomycetes, and Fungi Imperfection are included in the above list. It was initially planned to include a bacterium, Bacillus globisii, with the test organisms but this was not feasible as will be explained later. A few fungus species such as Alternaria solani were included because of the large amounts of data available on them as plant parasites and it was believed that these organisms would provide means of checking on techniques.

The conditions of relative humidity (R.H.), temperature, and time were preselected to establish limits based on experience of known conditions of materiel in storage. These were: (a) relative humidity—95, 90, 85, 80, 75, 70, 65, and 60 percent, (b) temperature—100°, 90°, 80°, 70°, 60°, and 50° F, (c) time—2, 4, 8, 16, and 32 days.

Each microorganism was first evaluated at the highest temperature and humidity for the shortest period of time. The investigation was then continued in sequence at successively lower temperature and relative humidity values until one or more variables became limiting.

The viability of spores of selected fungi was determined by employing cultures made from transfers not over 18 days old and in an actively growing, heavily sporulating condition. The spores used in such viability tests were not separated from vegetative structures. These tests were performed by placing spores on glass slides coated with a very thin transparent layer of an agar medium containing mineral salts and dextrose or other material which would not inhibit germination. The purpose of the layer was merely to hold spores in place. Individual tests were so conducted that temperature, humidity, or time was the only limiting factor. For example, biotin was added to the medium used in the evaluation of Memoniella, since the organism may need a trace of this vitamin for normal spore germination.

A minimum germination test for each condition was conducted by counting the number of germinating spores in 100 spores observed in not less than two microscope fields, at a magnification no less than 450%, on a single slide. Any evidence of an improper test was considered as sufficient reason for repeating the test.

#### **APPARATUS**

#### Humidity Chambers

Three humidity chambers of different sizes were used. Four cells, each 15 inches long, 9 inches high, and 5.5 inches wide, were constructed of Lucite plastic sheet with the tops made to inset the plastic boxes and thus fit tightly (figures 1 and 2). Three additional cells were constructed for use in a circular Warburg respirometer bath. These were similar to the rectangular chambers except that they were cut on a curved pattern to fit into the circular water bath (figures 3 and 4). Thirteen additional chambers were constructed by cutting the tops from glass carboys and thus making circular jars 12 inches in diameter and 15 inches tall (figures 5, 6, and 7). The edges of the jars were smoothed and foam rubber was used to

make a seal when the lid and a weight were placed on each jar. Each cell held 10 to 13 trays made of heavy aluminum screen. In the plastic cells, these trays held approximately 150 slides. The lids of all cells were drilled to permit the insertion of a fan shaft and wires for wet and dry bulb thermocouples. The bearings for the motor shafts were made of nylon rods to decrease frictional heat, and the one-hundredth horsepower motors which drove the fans were mounted 4 inches above the cells on wooden legs to further decrease the effective radiated heat. To insulate the lids, asbestos paper was glued to the top of the plastic lids and then covered with heavy aluminum foil.

Temperature within the cells would rise 3° to 4° F when the fans had steel motor shafts; but when the shafts were cut and the pieces coupled with lengths of plastic tubing, there was no measurable temperature rise. Therefore, this type of coupling was employed with all fan motors.

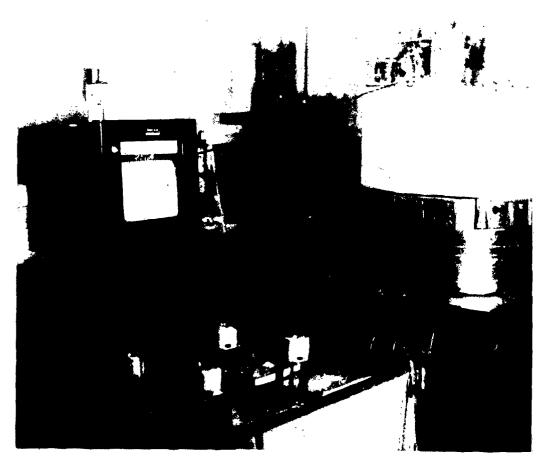


Figure 1. View of Constant Temperature Units and Brown Potentiometer

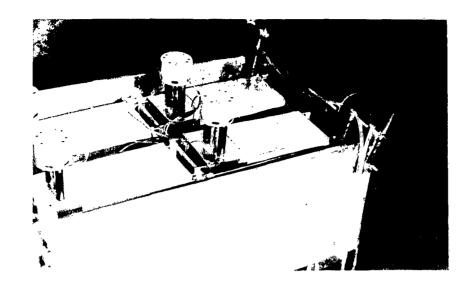


Figure 2. Cooling Unit Tank with Four Plastic Cells

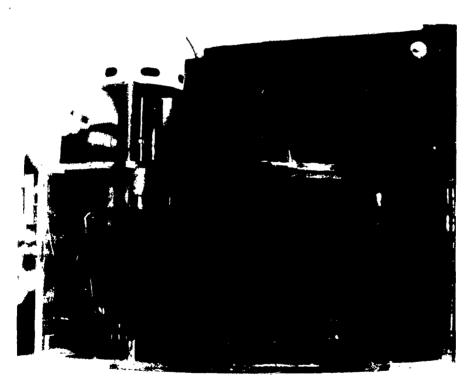


Figure 3. Side View of Unit Cell Shows Fan, Motor, Split Motor Shaft Joined by Tygon Tubing, Wet-Bulb Thermocouple Well, Perforated Aluminum Tiers Separated by Plastic Bumpers



Figure 4. Warburg Tank with Controls and Two Curved Plastic Cells for Work at Temperatures Above or Below Room Temperatures

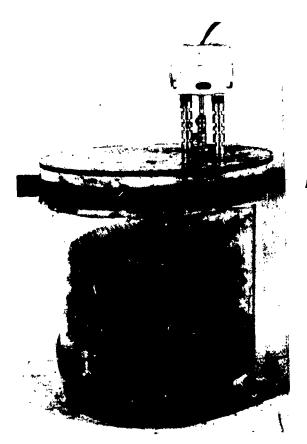


Figure 5. Cylindrical Glass Cell with Plastic Lid

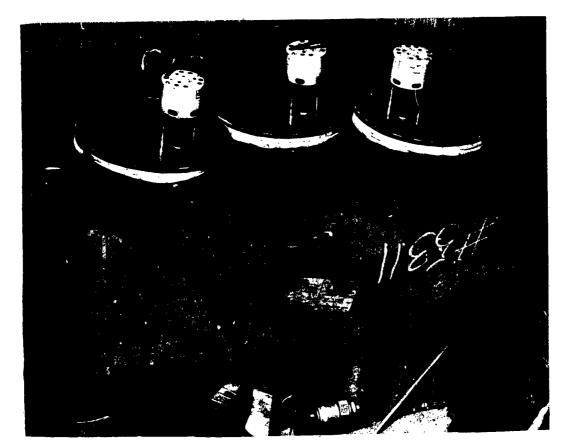


Figure 6. Cooling Unit with Three Glass Cells

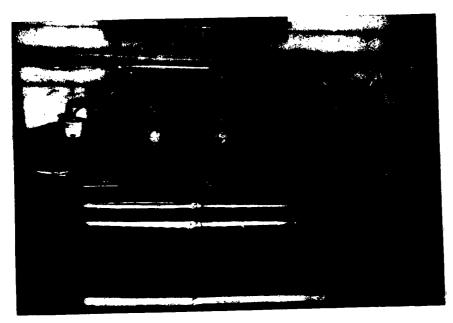


Figure 7. Large Metal Tank with Ten Glass Cells

#### Temperature Control

Temperature was controlled in the cells by immersion in constant temperature water baths. One was a Warburg respirometer bath with provision for heating and cooling, but this bath was small and could accommodate only three plastic cells. Two were rectangular, stainless steel photographic processing tanks with cooling coils; these were used for constant temperature baths at temperatures below room temperature. A fourth bath was used later to speed the gathering of data. This was a large, rectangular, steel tank approximately 6 feet long, 2 feet high, and 2.5 feet wide which held 10 of the large glass cells. Although this large tank had no heating or cooling facilities, it was maintained at below normal room temperature by placing in a constant temperature room. Temperatures in the various cells were determined by means of thermocouples attached to a 16-point recording Browm (Minneapolis-Honeywell) potentiometer which was checked at intervals against a retested thermometer graduated in one-tenth units.

It was found that during cold weather the temperature of the baths decreased and, to maintain constant temperature in all kinds of weather, an insulated room was constructed around three of the baths. A thermostatically controlled electric heater with fan was used to maintain the air temperature at 90° F and under these conditions water bath temperatures remained constant. The large water bath was maintained in a separate room insulated with 2-inch styrofoam sheeting and cooled with a thermostatically controlled, single-horsepower refrigeration unit. This bath, containing several hundred gallons of water, was completely free of cyclic fluctuations. All facilities for recorded experiments were maintained within ± 1° F.

#### Humidity Control

In earlier experiments saturated salt solutions were placed in the bottoms of the cells to maintain constant humidity conditions, but in certain instances the humidity measured did not agree with stated handbook values. Furthermore, it was difficult to find salt solutions providing the exact values desired at the lower ranges of relative humidity. Hence, glycerol solutions were substituted for salt solutions to obtain the desired relative humidity values. The use of glycerol solutions instead of salt solutions provided two advantages: (1) lack of corrosion and (2) a decrease in the tendency for relative humidity to fluctuate with temperature. According to calculations, at 98% R.H. a shift in temperature of 90° F would result in a change of only 0.5% R.H. and at 50% R.H. a similar shift in temperature would result in a change of only 3.2% R.H. Thus, any small temperature fluctuation would result in a negligible shift in R.H. In the rectangular cells, 800 ml of glycerin was added; in the Warburg cell, 650 ml; and in the glass jars, 1200 ml. Such amounts resulted in glycerin solutions 1.5 and 2.0 inches deep in each cell. With this amount of glycerin solution, it would require about 26 hours for a cell at 95% R.H. opened to a 50% R.H. atmosphere to change its relative humidity as much as 0.5%.

The glycerin solutions were prepared according to ASTM standard methods.\* In table I are listed the concentrations yielding the relative humidities of interest in this investigation. Results of tests, checking these solutions with wet and dry bulb thermocouples and an electric hygrometer, indicate that the solutions are within 2% of the expected relative humidities. Actually, because of instrumental errors, the expected relative humidities are probably more nearly correct. However, the measurements provide evidence that the humidities obtained definitely do not deviate from expected values by more than 2%.

#### GLYCERIN SOLUTION

The solutions were prepared in distilled water using a good industrial grade of glycerin (high gravity and dynamite grades are satisfactory). The concentration in terms of refractive index at 77° F for the desired relative humidity at any temperature between 32° F and 158° F may be calculated as follows:

$$(R_1 + A)^2 = (100 + A)^2 + A^2 - (H + A)^2$$
  
 $R_1 = 715.3 (T - 1.3333)$ 

where:

 $A = 25.60 - 0.1950T + 0.0008T^2$ 

H = relative humidity in percent

R = refractive index of the glycerin solution

T = temperature of solution in degrees Centigrade

The glycerol solutions listed in table I will yield the desired relative humidity with an accuracy of  $\pm$  0.2 in percentage at a temperature of 77° F. At other temperatures the error, if any, may increase with the deviation of the temperature from 77° F. The relative humidities associated with different refractive index values are presented in table I. The refractive indices for intermediate relative humidities and temperatures may be obtained by interpolation on a curve plotted from the tabular values or by calculation using the formula listed above.

<sup>\*</sup> Maintaining Constant Relative Humidity by Means of Aqueous Solutions, ASTM Standards, Part 6, 961, 1958.

TABLE I

PREPARATION AND TESTS OF GLYCEROL SOLUTIONS
FOR CONTROL OF RELATIVE HUMIDITY

Expected Relative Humidity (77° F)	Refractive Index	Percent Glycerol (by wt.)	Grams Glycerol per Liter Solution	Measured R.H. (at 60° F) with Thermocouples	Measured R.H. (at 60° F) with Electric Hygrometer
98.0	1.3463	11.25			
96.0	1.3560	18.80			
95.0	1.3602	22.0	231.50	94	93
90.0	1.3773	34.90	378.92	91	88
85.0	1.3905	44.72	497.33	84.	83
80.0	1.4015	52.30	592.34		79
75.0	1.4109	58.61	675.14		74
70.0	1.4191	64.15	747.43		71
65.0	1.4264	69.05	813.89		66
60.0	1.4329	73.40	873.86		
55.0	1.4387	77.30	928.46		
50.0	1.4440	80.65	976.03		
40.0	1.4529	86.30			

#### Aqueous Glycerin Solutions

A refractomete. was used for measurements in the range of refractive indices from 1.33 to 1.47 (sodium) with an accuracy of 0.0003.

About 0.1% by weight of copper sulphate was added to the glycerin solutions to prevent fungus growth. The most convenient way to measure the copper sulphate is to add 4 drops of saturated solution to each 100 ml of glycerin solution.

In preliminary work, thermocouples were used to measure temperature and relative humidity. A glass well containing distilled water was placed in each cell and a thermocouple with a wick was then placed (through a small hole in the foot of the well) with its tip in the well. At lower relative humidity values, the water evaporated from the well so rapidly and had such an influence on the relative humidity of the cell (because of the small volume of air in the cell) that the wet bulb method could not be used with accuracy. For this reason an electric hygrometer was used to measure relative humidity. Although this instrument was accurate only to 2% relative humidity, it was sufficiently accurate to determine if error had occurred in preparing the solutions.

#### MTCOLOGICAL TECHNIQUES

#### Organisms Used

Twenty-one different fungi were used. Considerable work was done with Bacillus globigi but it was so different from the other test organisms that it could not be used in conjunction with them. The spores of Bacillus globigii are much smaller than the fungus spores and consequently are much more difficult to observe and count under the microscope. Furthermore, Bacillus globigii spores differ from fungus spores in that, when they germinate, they do not do so by means of long germ tubes which grow out from the spores. The bacterial spores produce, on germination, bacterial cells which are approximately the same size and shape as the spores. Hence, it is almost impossible to determine by observation if germination has occurred.

#### Germination Techniques

Nutrient agar for use on slides was prepared with 0.5% malt extract in 0.75% agar. One drop of this medium was placed in the center of each microscope slide and allowed to dry. The relative humidity of the small transfer room where the slides were prepared was fairly low. Hence, a minimal time elapsed in the drying of the agar, forming a film on which spores were placed for germination.

After the agar was dry each slide was inoculated from a spore suspension prepared from cultures 14 to 16 days old. Spore suspensions were prepared by transferring spores from a culture tube by means of a sterile loop and/or sterile dissecting needle to a tube containing 1 ml of sterile distilled water. When necessary (depending upon the quantity of spores developed by a given organism) the suspension was diluted according to standard dilution methods until, upon checking, one microscope field contained not less than 100 spores.\*

This spore suspension was transferred to the dry agar on the slides with a loop. In most instances one loop contained sufficient spores to conduct the germination determinations according to specifications. The slides were carefully scanned for uniform spore distribution.

The inoculated slides were then placed within the cells of the constant temperature apparatus. Six replicate slides were prepared with each organism at each condition of temperature and humidity. Each slide had two inoculations and so twelve observations were made at each reading. All slides were read at intervals of 2, 4, 8, 16, and 32 days.

<sup>\*</sup> The tube dilution method is a variation suggested by:
Riker A. J., and Riker, R. S., "Introduction to Plant Diseases,"
Riker & Riker Co., Madison, Wisconsin, 1936
and
Smith, G., "Industrial Mycology," Arnold and Co., London, 1946

#### PRELIMINARY EXPERIMENTS

In addition to the temperature fluctuation already noted, another problem arose during the preliminary experiments. Germination readings for slides after 2 days were higher than slides after 4 days in the high humidity cells in the 100° F water bath. In these preliminary trials the slides which were read were removed and replicate slides were read on subsequent days. Slides on which germinating spores were counted at 2 days were always on the top tray of the cell, whereas the slides to be read at a later time were on lower trays. It was then observed that water was condensing on the inside surfaces of the lids of the cells and the presence of this condensate resulted in an abnormally high humidity in the proximity of slides on the top tray. Condensation resulted from the fact that the cells, although immersed in a water bath, were not completely submerged and the top inch of each cell was exposed to room air which was 10° F lower than the temperature of the water in which it was immersed. Thus, the cooler lid provided a surface on which moisture would condense, especially in cells maintained at high relative humidities. Abnormally high humidities near the lids not only caused rapid germination of the spores but had no relation to the average humidities of the cells. This localized condition of high humidity could neither be corrected by circulation of air within the cells nor by covering both the tops and bottoms of lids with asbestos paper lined with aluminum foil. The most effective method found for eliminating condensate was to heat the lids to raise them to the same temperature as those of the water baths. Small night-light fixtures with 7-watt bulbs were placed on the lids. Voltages to the lamps were adjusted by rheostat to the point at which there was sufficient heat to prevent condensation but not enough to heat the cells. This was determined by making temperature recordings within the cells. When the lamps received 100 volts, they emitted just enough heat to remove the condensate. Under these conditions, condensate occurred only in the 100° F bath where the temperature of the water was above the temperature of the room.

#### Uniformity

To determine the degree of uniformity of the conditions that existed in all parts of the cell, wet and dry thermocouples were placed at different positions within the cell. When the ends, sides, top, and bottom of the cell were checked, temperature readings in all positions were within 0.25° F of each other. To check further on uniformity of conditions, series of replicate slides were placed on all trays and in all positions in the cell. The fan was placed in the upper left-hand corner of the cell. In table II the slides numbered 1 were closest to the side of the cell which contained the fan, while slides numbered 6 were on the opposite side. Tray number 1, which was at the top of the cell, was closest to the fan whereas tray number 10 at the bottom of the cell was farthest from the fan. The results which were obtained from the 60 replicate slides in this series are presented in table II and illustrate the normal variation due to the organisms and technique. However, they do not indicate that there are any differences in germination resulting from the positioning of slides in the cells. Therefore, conditions within the cell may be considered uniform.

TABLE II

TEST OF THE UNIFORMITY OF CONDITIONS IN THE TEST CELL

60 Replicate Slides of Penicillium ochrochloron at 76% R.H. and 100°F

			Ger	ninatio	n perc	entage	s per f	<u>ield</u>				
	F	DAYS ield		DAYS eld		DAYS eld	16 D <b>Fi</b>	<b>e</b> ld		DAYS eld		DAYS eld
	<u>A</u>	<u>B</u>	<u> </u>	<u>B</u>	<u>A</u>	<u>B</u>	<u>A</u>	<u>B</u>		<u>B</u>	<u>A</u>	<u>B</u>
TRAY 1 Slide 1 2 3 4 5 6 Average	0 6 0 0 3 1	1 0 2 0 0 2	0 2 0 1 2 4	0 1 1 1 3 0	0 4 4 7 6 6	0 0 1 4 0 3	13 7 8 13 6	5 3 7 8 6 4	8 9 10 13 7 12	1 5 5 7 10 10 6	22 12 95 31 30 22	35 16 79 43 21 17
			-			•	•	•		•	•	
TRAY 2 Slide 1 2 3 4 5 6	1 0 2 0 0	0 0 2 0 0	2 0 0 1 0	0 1 2 1 0	5 3 4 1 0 4	11 3 0 6 0	6 6 11 9 4 6	4 3 11 7 5	68 10 12 10 6	11 5 16 13 7 6	19  31 35 17 45	27  46 39 32 47
Average		0.5		0.5		4		5		14		4
TRAY 3 Slide  1 2 3 4	0 0 0	0 0 2 0	0 1 2 3	0 0 2	3 0 7	4 2 2	10 7 6	7 11 5	12 8 12	9 14 7	36 17 23	15 12 26
4 5	3	0	1	7 0	15 7	6 3	16 14	9 10	20 15	10 13	20 34	16 19
5 6	ī	Ö	ō	1	ó	ĭ	7	3	9	10	46	30
Average		0.5				4		9		12	2	
TRAY 4 Slide												
1 2 3 4 5	1 3 2 0 0	0 0 1 0	2 2 0 0	5 0 1 1 2	9 6 1 4 4	5 1 2 3 0	11 7 13 13	5 2 6 7 11	12 8 9 17 13	14 9 16 10	43 22 20 85	32 17 31 20
•	<u> </u>			<del></del>						21	24	26
Average		0.5	1	•	;	3	,	7		17	3	3
TRAY 5 Slide	•	^	7	4	ae.	2		10	30	10	0.5	1.0
1 2 3 4 5	0 1	0 0	7 5	6 5	25 11	2 14	57 10	13 10	72 12	18 10	95 12	17 8
3	1	0	1	2	3	1	9	2	10	7	22	18
4	4	3	3	2 6	6	6	39	18	42	20	13	35
5	0	0	0	3 3	4	0	5	0	6	3	19	15
	2	1	_4	3_	<u>13</u>	3_	12		11	16	47	<u>75</u>
Average		1	4	4		7		15	1	.9	3	1

TABLE II (Continued)

	2 D	eld		DAYS		DAYS .eld		DAYS eld		DAYS eld	32 D Fie	1 <b>d</b>
	<u>A</u>	<u>B</u>	_A	В	<u>A</u>	В	<u>A</u>	<u>B</u>	<u>A</u>	<u>B</u>	<u>A</u>	<u>B</u>
TRAY 6												
1 2	0	1	10	7	16	3	23	14	7	19	10	9
	0	0	4	6	5	6	12	19 6	18	29	17	16
3 4	3 2	0 0	0 4	4 5	4 12	6 5	12 14	6	15	16	13	21
4 5	3	1	3	7	2	8	5	7	10	13	16	25
5 6	2	ō	3	3	13	4	6	10	8	12	25	11
Average		1		5		7		1	1		1	
	•	•				•	_	-	_	_	_	-
TRAY 7 Slide 1	1	0	2	0	3	7	35	13	16	11	21	74
2	3	1	4	6	8	6	28	14	30	18	28	36
3	2	ō	3	2	13	6	8	20	5	17	13	17
4	ō	2	3	ō	5	8	10	9	16	12	21	16
5	3	0	6	2	4	0	6	2	7	10		
6	_5	1_	_5	0	10	3	9	12	4	7	17	20
Average	:	2		2		6	1	.4	1	3	2	6
TRAY 8												
Slide	_	_	_			•	_	,	•	-	26	13
1	2	0	2 2	1	4	2 6	5 6	4 10	8 9	7 9	<b>3</b> 6 8	13
2	6 1	1 0	0	3 4	1 3	11	12	18	9	12	15	21
<b>3</b> 4	2	1	2	2	6	4	7	7	5	12	25	11
5	5	ō	3	2	3	3	ģ	4	15	6	17	32
5 6	Õ	ī	_1	11	4	7_	12	13	16	10	15	23
Average		2		2		5		9	1	0	1	9
TRAY 9												
Slide												
1	1	0	0	1	2	1	12	3	6	14	35	17
2	3	0	4	0	7	0	9	4	15	8	13	16
3	1	0	0	0	3	4	23	6 4	20 6	9 12	36 13	28 19
4	4	1 0	2	1 1	4 3	7 7	10 10	6	14	5	16	27
5	0	0	0	_	0	4	7	5	7	3		
6 Average	<u> </u>	1		1		4	<del></del>	8	1	.0	2	2
TRAY 10												
Slide												
1	0	0	0	0	1	9	8	4	13	5	22	16
1 2	1	0	1	0			9	4	10	19	35	21
3	2	3	3	3			5	1	12	10	14	11
4	ŋ	0	1	1			1	2	11	6	42	20
3 4 5 6	2	1	3				1	4	4	8	26	15
6	0	3_	_1	1								
Average		1		1		5		3		8	1	9

#### Reproducibility

In addition to determining if there was uniformity of conditions at different parts of the cell it was necessary to determine if similar results could be obtained in different runs, thus providing a measure of the reliability of the testing technique. In table III are presented results obtained at the end of 32 days in two separate experiments. Three different organisms were used and results obtained in the separate experiments were quite similar. Thus, the procedure used yields reproducible results.

### TABLE III COMPARISON OF DATA FROM IDENTICAL RUNS TO INDICATE THE REPRODUCIBILITY OF RESULTS BY THE TESTING TECHNIQUE

Organism		R.H. Run 2	85% Run 1	R.H. Run 2		R.H. Run 2		R.H. Run 2
Aspersillus tamarii	88	65	8	6	24	56	4	23
Aspergillus niger	96	100	3	<b>2</b> <sup>-</sup>	96	100	3	1
Trichoderma species	63	85	8	3	78	67	20	7

#### DISCUSSION

Data concerning percentage of germination in two fields of each of six replicate slides were collected for each of the test organisms at the specified conditions of relative humidity and temperature. The average germination percentages for each organism at each temperature and each relative humidity are presented in tables IV to XXXIII.

The conditions under which there was no spore germination of any of the 21 test fungi during a 32-day test period were as follows:

- (1) 100° F at 65, 60, and 55% R.H. (2) 80° F at 70, 65, and 60% R.H. (3) 70° F at 65 and 60% R.H.

- 60° F at 70, 65, 60, and 55% R.H.
- 50° F at 55 and 50% R.H.

Tables XXIV and XXXV show the effects of temperature and relative humidity on germination. As the temperature was lowered from 100° F and since the testing period was limited to 32 days, no statement may be made concerning the capacity of spores to germinate over longer periods of time at lower temperatures. It may be observed that, at 100° F and (with only one exception) at 80° F, there was almost complete spore germination of all of the 21 fungi at 95% R.H. in 32 days. It may be inferred therefore that at 100° F and 80° F temperature is not limiting for 32 days.

In 32 days at a temperature of 100° F, the spores of some organisms germinated under relative humidity conditions as low as 70% but none germinated under conditions of lower relative humidity during this time period. While spores of some organisms failed to germinate at 70% and higher R.H., none were capable of germination at 65% R.H. and lower. Therefore, the minimum value for spore germination of all 21 fungi at 100° F was between 65 and 70% R.H. At 80°, 70°, and 60° F there was little or no germination at 70% R.H. Many did not begin to germinate until the relative humidity was raised to 80%.

From the tables we can see that at 75% R.H. there is no increase in the degree of germination. At all temperatures only 2 of the 21 organisms had germination percentages greater than 3 at 75% R.H. and for the great majority there was no germination at all. The most striking observation was that at all temperatures there was a great increase in number of spores of different fungi germinating at 80% R.H. that had failed to germinate or did so only sparsely at 75% R.H. It may be concluded that, although germination of some fungus spores may occur at 70 to 80% R.H., fungal deterioration under equilibrium conditions might not be expected to become a serious problem in less than 3 months at these humidities. The reservation on "equilibrium conditions" must be considered since, under fluctuating environmental conditions, condensation of water may occur, thus providing temporarily wet surfaces which would support unusually rapid fungus growth. Under natural conditions fluctuating rather than constant temperature and humidity are the rule. Therefore, in practice, fungus growth might be serious at "average" relative humidities below 80%, whereas it would not be so under constant conditions.

According to the computed data in table XXXIV, the effect of temperature lowering (in the range of 100° F to 50° F) on germination of spores is to reduce the rate of germination but not to prevent this process. At lower temperatures the percentage of germination is lower than at higher temperatures at the same R.H. at any given time up to 32 days. If the tests were extended to 6 months the overall germination at 85% R.H. for 100° F might, for example, be the same as for 60° F. However, for short periods of time germination is inhibited by reduction in temperature—a benefit which is derived mainly at the lower relative humidities. At 90% R.H. and 95% R.H. the differences are limited to only a few days. At these high humidity values, even 50° F is not a serious deterrent to fungus growth.

Among the less expected results of these experiments is the uniformity of results obtained with different fungi. The majority of the organisms employed reacted similarly to conditions of temperature and relative humidity. None seemed unusually well suited to arid conditions and none were appreciably inhibited at either of the temperature extremes.

Some of the test fungi yielded more dependable and reproducible results, while others seemed to be erratic. Generally poor germination was observed for Myrothecium verrucaria and Memnoniella echinata. Pullularia pullulans, Botrytis cinerea, and Rhizopus stolonifer were somewhat erratic but Cladosporium fulyum, Monilia brunnea, Curvularia lunata, Penicillium funiculosum, and Aspergillus nidulans yielded fairly consistent dependable results and seem well suited for work of this type.

#### CONCLUSIONS

The germination of spores of all 21 fungi used in this investigation was prevented at approximately 70% R.H., regardless of temperature. Therefore, Air Force material stored at constant relative humidities not exceeding 70% R.H. would probably be free of fungus deterioration. However, with fluctuating temperatures and average (but not constant) relative humidities near this value, fungus deterioration may not necessarily be prevented because of localized moisture condensation.

Whereas 70% R.H. may be considered the cut-off point for mold spore germination, the overall germination of most of the fungi was so low between 70 and 80% R.H. that, over relatively short periods of time (6 months or less), storage at these humidities should create no serious problem. Fungus deterioration might be expected to become an important problem when the relative humidity is 80% or greater.

Within the limits of the temperature range used in these tests, low temperature retarded but did not prevent fungus spore germination. This may be considered a practical deterrent at the lower relative humidities (below 90% R.H.) for brief periods of storage, but at 90% R.H. or higher the retardation period is too short to be of practical value.

TABLE IV

AVERAGED RESULTS OF SPORE GERMINATION TESTS

100°F and 95% R.H.

Curvularia lunata

TABLE V  $\begin{tabular}{llll} AVERAGED & RESULTS & OF & SPORE & GERMINATION & TESTS \\ \hline $100^\circ F$ & and & 90\% & R.H. \\ \end{tabular}$ 

Organism	2 days	4 days	8 days	16 days	32 days
Pullularia pullulans	0	12	19	87	99
Helminthosporium sp.	2	14	15	62	100
Alternaria solani	3	25	39	92	100
Memnoniella echinata	3	10	32	92	98
Penicillium citrinium	29	87	91	100	100
Aspergillus glaucus	4	24	95	100	100
Cladosporium fulvum	0	0	0	27	90
Rhizopus arrhizus	2	14	36	44	89
Monilia brunnea	0	0	7	46	84
Rhizopus stolonifer	32	86	100	100	100
Aspergillus fumigatus	0	8	64	100	100
Penicillium funiculosum	14	37	76	100	100
Aspergillus nidulans	43	95	99	100	100
Aspergillus niger	17	72	<b>30</b>	99	100
Trichoderma sp.	0	3	61	76	100
Myrothecium verrucaria	0	0	1	63	74
Aspergillus tamarii	17	84	88	100	100
Chaetomium globosum	22	80	98	100	100
Penicillium ochrochloron	23	89	100	100	100
Botrytis cinerea	0	0	19	71	100
Curvularia lunata	2	9	42	100	100

TABLE VI

AVERAGED RESULTS OF SPORE GERMINATION TESTS

### 100°F and 85% R.H.

Organism	2 days	4 days	8 days	16 days	32 days
Fullularia pullulans	0	0	0	1	16
Helminthosporium sp.	0	0	2	45	100
Alternaria solani	0	11	17	68	85
Memnoniella echinata	0	0	25	91	98
Penicillium citrinium	0	o	16	67	78
Aspergillus glaucus	0	2	13	75	100
Cladosporium fulvum	0	0	0	0	26
Rhizopus arrhizus	0	4	22	80	94
Monilia burnnea	0	0	0	12	34
Rhizopus stolonifer	9	15	27	37	55
Aspergillus fumigatus	0	0	16	92	100
Penicillium funiculosum	0	0	10	0	98
Aspergillus nidulans	5	30	53	98	100
Aspergillus niger	1	12	21	64	90
Trichoderma sp.	0	0	0	4	21
Myrothecium verrucaria	0	0	1	5	28
Aspergillus tamarii	3	21	80	91	100
Chaetomium globosum	0	5	19	82	100
Penicillium ochrochloron	0	0	0	44	100
Botrytis cinerea	0	0	0	2	79
Curvularia lunata	1	3	5	28	78

TABLE VII

AVERAGED RESULTS OF SPORE GERMINATION TESTS

100°F and 80% R.H.

Organism	2 days	4 days	8 days	16 days	32 days
Pullularia pullulans	0	0	0	0	0
Helminthosporium sp.	0	9	10	15	32
Alternaria solani	0	0	0	0	0
Memnoniella echinata	0	0	0	0	1
Penicillium citrinium	0	0	0	2	8
Aspergillus glaucus	0	55	100	100	100
Cladosporium fulvum	0	0	22	27	48
Rhizopus arrhizus	0	0	0	12	42
Monilia burnnea	0	3	3	6	23
Rhizopus stolonifer	0	1	17	29	65
Aspergillus fumigatus	0	0	15	61	100
Penicillium funiculosum	0	0	0	0	69
Appergillus nidulans	0	0	5	11	50
Aspergillus niger	0	0	58	83	90
Trichoderma sp.	0	0	0	5	18
Myrothecium verrucaria	0	0	0	0	0
Aspergillus tamarii	0	3	40	79	99
Chaetomium globosum	0	0	2.	3	8
penicillium ochrochloron	0	0	0	0	1
Botrytis cinerea	0	0	0	0	2
Curvularia lunata	0	0	4	5	9

TABLE VIII

AVERAGED RESULTS OF SPORE GERMINATION TESTS

## 100°F and 75% R.H.

Organism	2 days	4 days	8 days	16 days	32 days
Pullularia pullulans	0	0	0	0	0
Helminthosporium sp.	1	4	8	13	23
Alternaria solani	0	0	0	0	0
Memonoiella echinata	0	0	0	0	0
Penicillium citrinium	0	0	c	0	0
Aspergillus glaucus	0	0	0	0	3
Cladosporium fulvum	o	0	1	1	2
Rhizopus arrhizus	0	0	0	0	0
Monilia brunnea	o	0	0	0	0
Rhizopus stolonifer	0	2	3	4	9
Aspergillus fumigatus	0	1	1	2	2
Penicillium funiculosum	0	0	0	0	0
Aspergillus nidulans	0	0	0	0	1
Aspergillus niger	0	0	0	0	0
Trichoderma sp.	0	0	0	0	0
Myrothecium verrucaria	0	0	0	0	0
Aspergillus tamarii	0	0	0	0	3
Chactomium globosum	0	0	0	0	1
Penicillium ochrochloron	0	0	0	0	0
Botrytis cinerea	0	0	0	0	0
Curvularia lunata	0	0	0	o	0

TABLE IX  $\begin{tabular}{lllll} AVERAGED & RESULTS & OF SPORE & GERMINATION & TESTS \\ \hline 100°F & and & 70% & R.H. \\ \end{tabular}$ 

Organism	2 days	4 days	8 days	16 days	32 days
Pullularia pullulans	0	0	0	0	0
Helminthosporium sp.	0	5	7	14	32
Alternaria solani	o	0	0	0	0
Memnoniella echinata	0	0	0	1	2
Penicillium citrinium	0	0	0	0	0
Aspergillus glaucus	o	0	1	1	1
Cladosporium fulvum	0	0	0	0	1
Rhizopus arrhizus	0	0	0	0	0
Monilia brunnea	0	1	1	1	2
Rhizopus stolonifer	0	2	3	4	5
Aspergillus fumigatus	0	0	0	0	2
Penicillium funiculosum	0	0	0	0	0
Aspergillus nidulans	0	0	0	0	0
Aspergillus niger	0	0	0	0	0
Trichoderma sp.	0	0	0	0	0
Myrothecium verrucaria	0	0	0	0	0
Aspergillus tamarii	0	0	0	0	1
Chaetomium globosum	0	0	0	0	2
Penicillium ochrochloron	0	0	0	0	0
Botrytis cinerea	0	0	0	0	0
Curvularia lunata	0	0	0	0	1

TABLE X

AVERAGED RESULTS OF SPORE GERMINATION TESTS

80°F and 95% R.H.

Organism	2 days	4 days	8 days	16 days	32 days
Pullularia pullulans	6	57	100	100	100
Helminthosporium sp.	6	27	90	100	100
Alternaria solani	15	26	52	100	100
Memmoniella echinata	28	61	100	100	100
Penicillium citrinium	29	69	100	100	100
Aspergillus glaucus	32	87	100	100	100
Cladosporium fulvum	0	57	100	100	100
Rhizopus arrhizus	3	62	100	100	100
Monilia brunnea	48	90	100	100	100
Rhizopus stolonifer	33	55	85	100	100
Aspergillus fumigatus	5	40	100	100	100
Penicillium funiculosum	10	54	93	100	100
Aspergillus nidulans	12	<b>3</b> 5	60	100	100
Aspergillus niger	41	82	100	100	100
Trichoderma sp.	21	49	100	100	100
Myrothecium verrucaria	2	5	14	42	62
Aspergillus tamarii	16	45	100	100	100
Chaetomium globosum	36	71	100	100	100
Penicillium ochrochloron	5	56	100	100	100
Botrytis cinerea	10	32	87	100	100
Curvularia lunata	17	46	100	100	100

Organism	2 days	4 days	8 days	16 days	32 days
Pullularia pullulans	3	6	<b>3</b> 5	79	100
Helminthosporium sp.	1	9	34	100	100
Alternaria solani	0	10	27	100	100
Memnoniella echinata	11	17	34	100	100
Penicillium citrinium	7	40	79	100	100
Aspergillus glaucus	0	20	50	100	100
Cladosporium fulvum	0	24	83	100	100
Rhizopus arrhizus	0	0	32	100	100
Monilia brunnea	0	0	7	15	80
Rhizopus stolonifer	27	45	69	100	100
Aspergillus fumigatus	2	12	45	96	98
Penicillium funiculosum	4	6	35	100	100
Aspergillus nidulans	2	11	59	100	100
Aspergillus niger	11	34	77	100	100
Trichoderma sp.	7	16	63	100	100
Myrothecium verrucaria	1	3	5	10	43
Aspergillus tamarii	6	50	85	100	100
Chaetomium globosum	6	13	34	71	100
Penicillium ochrochloron	3	18	40	100	100
Botrytis cinerea	0	0	19	100	100
Curvularia lunata	0	4	13	100	100

TABLE XII

AVERAGED RESULTS OF SPORE GERMINATION TESTS

### 80°F and 85% R.H.

Organism	2 days	4 days	8 days	16 days	32 days
Pullularia pullulans	0	0	0	0	0
Helminthosporium sp.	0	0	0	3	10
Alternaria solani	0	0	4	15	30
Memnoniella echinata	0	0	0	0	0
Penicillium citrinium	0	12	39	95	100
Aspergillus glaucus	2	5	55	100	100
Cladosporium fulvum	0	0	3	25	100
Rhizopus arrhizus	0	1	7	15	45
Monilia brunnea	1	3	9	51	74
Rhizopus stolonifer	5	7	13	24	53
Aspergillus fumigatus	0	0	3	14	82
Penicillium funiculosum	0	0	8	27	87
Aspergillus nidulans	2	8	21	76	77
Aspergillus niger	0	3	8	29	66
Trichoderma sp.	0	6	10	56	100
Myrothecium verrucaria	0	0	0	0	4
Aspergillus tamarii	0	19	52	100	100
Chaetomium globosum	0	0	2	14	22
Penicillium ochrochloron	0	2	8	21	54
Botrytis cineres	0	0	2	8	32
Curvularia lunata	0	0	0	2	15

Organism	2 days	4 days	8 days	16 days	32 days
Pullularia pullulans	0	0	0	0	0
Helminthosporium sp.	0	0	0	0	0
Alternaria solani	0	0	0	0	0
Memmoniella echinata	0	0	0	0	0
Penicillium citrinium	4	7	17	46	61
Aspergillus glaucus	0	32	43	78	84
Cladosporium fulvum	0	0	0	7	49
Rhizopus arrhizus	0	0	0	0	2.
Monilia brunnea	0	0	0	9	15
Rhizopus stolonifer	0	0	0	1	14
Aspergillus fumigatus	0	0	0	0	41
Penicillium funiculosum	0	0	2	12	34
Aspergillus nidulans	0	0	0	0	63
Aspergillus niger	0	0	0	0	0
Trichoderma sp.	0	0	0	0	0
Myrothecium verrucaria	0	0	0	0	0
Aspergillus tamarii	0	0	0	0	7
Chaetomium globosum	0	0	1	6	11
Penicillium ochrochloron	0	0	0	0	18
Botrytis cinerea	o	0	0	5	8
Curvularia lunata	0	0	0	3	3

TABLE XIV

AVERAGED RESULTS OF SPORE GERMINATION TESTS

# 80°F and 75% R.H.

Organism	2 days	4 days	8 days	16 days	32 days
Pullularia pullulans	o	0	0	0	0
Helminthosporium sp.	0	0	0	0	0
Alternaria solani	0	0	0	0	0
Memnoniella echinata	0	0	0	0	0
Penicillium citrinium	0	0	0	0	0
Aspergillus glaucus	0	0	0	0	0
Cladosporium fulvum	0	0	0	0	0
Rhizopus arrhizus	0	0	0	0	0
Monilia brunnea	0:	0	0	0	2
Rhizopus stolonifer	0	0	0	0	0
Aspergillus fumigatus	0	0	0	0	0
Penicillium funiculosum	0	0	0	0	0
Aspergillus midulans	0	0	0	0	1
Aspergillus aiger	0	0	0	0	0
Trichoderma sp.	0	0	0	1	4
Myrothecium verrucaria	0	0	0	0	0
Aspergillus tamarii	0	0	0	0	3
Chaetomium globosum	0	0	0	0	5
Penicillium ochrochloron	0	0	0	0	0
Botrytis cinerea	0	0	0	0	o
Curvularia lunata	o	0	0	0	O

TABLE XV AVERAGED RESULTS OF SPORE GERMINATION TESTS  $70^{\circ}$ F and 95% R.H.

Curvularia lunata

TABLE XVI

AVERAGED RESULTS OF SPORE GERMINATION TESTS

70°F and 90% R.H.

TABLE XVII

AVERAGED RESULTS OF SPORE GERMINATION TESTS

70°F and 85% R.H.

Organism	2 days	4 days	8 days	16 days	32 days
Pullularia pullulans	0	0	1	5	15
Helminthosporium sp.	0	1	5	18	41
Alternaria solari	0	1	5	21	43
Memnoniella echinata	0	0	0	4	13
Penicillium citrinium	6	7	15	35	58
Aspergillus glaucus	2	5	22	61	82
Cladosporium fulvum	0	5	14	37	71
Rhizopus arrhizus	0	1	2.	3	34
Monilia brunnea	0	0	3	10	30
Rhizopus stolonifer	0	15	23	39	59
Aspergillus fumigatus	0	3	7	18	49
Penicillium funiculosum	0	9	19	45	100
Aspergillus nidulans	13	18	29	48	71
Aspergillus niger	0	9	14	27	46
Trichoderma sp.	5	7	23	55	100
Myrothecium verrucaria	0	0	6	10	19
Aspergillus tamarii	4	6	8	18	38
Chaetomium globosum	0	0	0	6	21
Penicillium ochrochloron	0	15	13	33	65
Botrytis cinerea	0	4	5	12	30
Curvularia lunata	0	0	0	12	34

TABLE XVIII

AVERAGED RESULTS OF SPORE GERMINATION TESTS

#### 70°F and 80% R.H.

Organism	2 days	4 days	8 days	16 days	32 days
Pullularia pullulans	o	0	0	7	23
Helminthosporium sp.	0	0	0	4	25
Alternaria solani	0	0	0	5	12
Memnoniella echinata	0	0	0	1	5
Penicillium citrinium	o	0	8	16	32
Aspergillus glaucus	4	5	9	16	52
Cladosporium fulvum	1	1	2	11	42
Rhizopus arrhizus	o	0	2	6	10
Monilia brunnea	0	1	2	5	11
Rhizopus stolonifer	0	11	15	32	44
Aspergillus fumigatus	0	0	3	9	24
Penicillium funiculosum	0	1	6	20	57
Aspergillus nidulans	5	8	18	36	57
Aspergillus niger	0	0	14	21	31
Trichoderma sp.	4	6	10	20	52
Myrothecium verrucaria	0	0	0	1	4
Aspergillus tamarii	2	4	7	13	30
Chaetomium globosum	0	0	0	2	9
Penicillium ochrochloron	0	8	13	30	62
Botrytis cinerea	0	0	0	7	19
Curvularia lunata	0	0	3	6	13

TABLE XIX

AVERAGED RESULTS OF SPORE GERMINATION TESTS

# 70°F and 75% R.H.

Organism	2 days	4 days	8 days	16 days	32 days
Pullularia pullulans	0	0	0	0	0
Helminthosporium sp.	0	0	0	0	0
Alternaria solani	0	-	0	0	0
Memnoniella echinata	0	-	0	0	0
Penicillium citrinium	0	0	0	1	4
Aspergillus glaucus	0	. 0	0	0	0
Cladosporium fulvum	0	0	0	0	0
Rhizopus arrhizus	0	0	0	0	0
Monilia brunnea	0	0	0	0	0
Rhizopus stolonifer	0	0	0	0	o
Aspergillus fumigatus	0	0	0	0	0
Penicillium funiculosum	0	0	0	0	1
Aspergillus nidulans	0	0	0	0	1
Aspergillus niger	0	0	0	0	0
Trichoderma sp.	0	0	0	0	0
Myrothecium verrucaria	0	0	0	0	0
Aspergillus tamarii	0	0	0	0	0
Chaetomium globosum	0	o	1	1	4
Penicillium ochrochloron	0	0	0	0	1
Botrytis cinerea	0	-	0	0	0
Curvularia lunata	0	-	0	0	o

TABLE XX

AVERAGED RESULTS OF SPORE GERMINATION TESTS

### 70°F and 70% R,H.

Organism	2 days	4 days	8 days	16 days	32 days
Pullularia pullulans	0	0	0	0	0
Helminthosporium sp.	0	0	0	0	1
Alternaria solani	0	0	0	0	0
Memnoniella echinata	0	0	0	0	0
Penicillium citrinium	0	-	-	-	0
Aspergillus glaucus	0	-	0	0	0
Cladosporium fulvum	0	-	0	0	0
Rhizopus arrhizus	0	-	0	0	0
Monilia brunnea	0	-	0	0	0
Rhizopus stolonifer	0	0	0	0	0
Aspergillus fumigatus	0	0	0	0	0
Penicillium funiculosum	0	0	0	0	1
Aspergillus nidulans	0	0	0	0	0
Aspergillus niger	0	0	0	0	0
Trichoderma sp.	0	0	0	0	0
Myrothecium verrucaria	0	0	0	0	0
Aspergillus tamarii	0	0	0	0	0
Chaetomium globosum	0	0	0	1	3
penicillium ochrochloron	0	0	0	0	0
Botrytis cinerea	o	0	0	0	0
Curvularia lunata	0	0	0	0	0

TABLE XXI

AVERAGED RESULTS OF SPORE GERMINATION TESTS

#### 60°F and 95% R.H.

Organism	2 days	4 days	8 days	16 days	32 days
Pullularia pullulans	0.	0	1	20	35
Helminthosporium sp.	0	3	29	92	96
Alternaria solani	25	91	100	100	100
Memnoniella echinata	0	5	30	96	100
Penicillium citrinium	0	18	96	98	100
Aspergillus glaucus	0	4	40	90	100
Cladosporium fulvum	2	21	41	97	100
Rhizopus arrhizus	0	2	6	9	23
Monilia brunnea	0	6	18	31	39
Rhizopus stolonifer	0	4	7	17	25
Aspergillus fumigatus	0	1	21	97	100
Penicillium funiculosum	0	5	9	65	86
Aspergillus nidulans	0	0	1	19	58
Aspergillus niger	10	35	87	97	99
Trichoderma sp.	0	1	3	17	46
Myrothecium verrucaria	0	0	4	6	35
Aspergillus tamarii	0	3	25	51	58
Chaetomium globosum	0	2	17	73	91
Penicillium ochrochloron	0	3	13	28	81
Botrytis cinerea	0	6	27	96	91
Curvularia lunata	0	1	2	10	

Organism	2 days	4 days	8 days	16 days	32 days
Pullularia pullulans	0	o	2	23	57
Helminthosporium sp.	0	1	7	18	31
Alternaria solani	6	33	58	95	100
Memnoniella echinata	0	o	13	41	50
Penicillium citrinium	0	7	40	100	100
Aspergillus glaucus	0	3	32	100	100
Cladosporium fulvum	0	9	26	78	91
Rhizopus arrhizus	0	1	8	15	21
Monilia brunnea	0	2	8	37	42
Rhizopus stolonifer	0	2	7	8	23
Aspergillus fumigatus	0	0	3	11	100
Penicillium funiculosum	0	1	7	36	44
Aspergillus nidulans	0	0	7	58	75
Aspergillus niger	2	15	29	80	80
Trichoderms sp.	0	0	9	41	74
Myrothecium verrucaria	0	0	1	22	29
Aspergillus tamarii	0	1	9	37	54
Chaetomium globosum	0	O	5	25	73
Penicillium ochrochloron	1	2	6	14	19
Botrytis cinerea	0	1	2	9	13
Curvularia lunata	0	o	0	23	26

TABLE XXIII

AVERAGED RESULTS OF SPORE GERMINATION TESTS

# 60°F and 85% R.H.

Organism	2 days	4 days	8 days	16 days	32 days
Pullularia pullulans	0	0	0	1	3
Helminthosporium sp.	0	0	8	12	27
Alternaria solani	0	5	18	51	56
Memnoniella echinata	0	0	0	8	10
Penicillium citrinium	0	0	4	9	15
Aspergillus glaucus	0	0	9	39	48
Cladosporium fulvum	0	0	11	20	36
Rhizopus arrhizus	0	0	0	15	19
Monilia brunnea	0	0	4	4	7
Rhizopus stolonifer	0	0	1	2	10
Aspergillus fumigatus	0	0	0	7	8
Penicillium funiculosum	0	0	0	24	44
Aspergillus nidulans	0	0	2	11	17
Aspergillus niger	0	0	4	17	30
Trichoderma sp.	0	0	5	7	9
Myrothecium verrucaria	0	0	0	0	1
Aspergillus tamarii	0	0	1	2	3
Chaetomium globosum	0	0	0	1	3
Penicillium ochrochloron	o	o	4	4	7
Botrytis cinerea	0	0	0	1	2
Curvularia lunata	0	0	2	5	7

TABLE XXIV

AVERAGED RESULTS OF SPORE GERMINATION TESTS

# 60°F and 80% R.H.

Organism	2 days	4 days	8 days	16 days	32 days
Pullularia pullulans	0	0	0	1	2
Helminthosporium sp.	9	9	2	8	14
Alternaria solani	0	1	8	44	63
Memnoniella echinata	0	0	0	2	5
Penicillium citrinium	0	0	1	2	5
Aspergillus glaucus	. 0	0	1	12	17
Cladosporium fulvum	0	0	3	17	20
Rhizopus arrhizus	0	0	0	5	8
Monilia brunnea	0	0	0	2	5
Rhizopus stolonifer	0	0	1	20	24
Aspergillus fumigatus	0	0	0	2	3
Penicillium funiculosum	o	0	2	15	22
Aspergillus nidulans	0	0	2	6	13
Aspergillus niger	0	0	0	9	12
Trichoderma sp.	0	0	0	2	3
Myrothecium verrucaria	0	0	0	1	3
Aspergillus tamarii	0	0	0	1	1
Chaetomium globosum	0	0	0	1	3
penicillium ochrochloron	0	0	0	1	3
Botrytis cinerea	0	0	1	8	12
Curvularia lunata	0	0	0	6	11

TABLE XXV

AVERAGED RESULTS OF SPORE GERMINATION TESTS

60°F and 75% R.H.

Organism	2 days	4 days	8 days	16 days	32 days
Pullularia pullulans	0	0	0	1	3
Helminthosporium sp.	0	0	0	0	0
Alternaria solani	0	0	0	0	0
Memnoniella echinata	0	0	0	0	0
Penicillium citrinium	. 0	0	0	0	0
Aspergillus glaucus	0	0	0	0	0
Cladosporium fulvum	0	0	0	0	0
Rhizopus arrhizus	0	0	0	0	0
Monilia brunnea	0	0	0	0	o
Rhizopus stolonifer	0	0	0	0	0
Aspergillus fumigatus	0	0	0	0	•0
Penicillium funiculosum	0	0	0	0	0
Aspergillus nidulans	0	0	0	0	0
Aspergillus niger	0	0	0	0	0
Trichoderma sp.	0	0	0	0	0
Myrothecium verrucaria	0	0	0	0	0
Aspergillus tamarii	0	0	0	0	0
Chaetomium globosum	0	0	0	0	0
Penicillium ochrochloron	0	0	0	0	0
Botrytis cinerea	0	0	0	0	0
Curvularia lunata	0	0	0	0	0

TABLE XXVI

AVERAGED RESULTS OF SPORE GERMINATION TESTS

50°F and 95% R.H.

			-		
Organism	2 days	4 days	8 days	16 days	32 days
Pullularia pullulans	12	14	17	28	63
Helminthosporium sp.	98	100	100	100	100
Alternaria solani	0	0	23	62	100
Memnoniella echinata	0	0	2	7	35
Penicillium citrinium	0	2	75	100	100
Aspergillus glaucus	0	33	100	100	100
Cladosporium fulvum	0	25	100	100	100
Rhizopus arrhizus	0	0	5	13	63
Monilia brunnea	0	0	0	11	60
Rhizopus stolonifer	0	6	97	100	100
Aspergillus fumigatus	0	0	0	41	95
Penicillium funiculosum	0	0	9	49	100
Aspergillus nidulans	0	0	4	27	62
Aspergillus niger	0	0	0	75	94
Trichoderma sp.	3	3	10	30	73
Myrothecium verrucaria	0	0	0	5	32
Aspergillus tamarii	0	0	4	34	97
Chaetomium globosum	0	10	84	100	100
Penicillium ochrochloron	0	0	1	38	100
Botrytis cinerea	0	8	100	100	100
Curvularia lunata	0	31	97	100	100

TABLE XXVII

AVERAGED RESULTS OF SPORE GERMINATION TESTS

50°F and 90% R.H.

Organism	2 days	4 days	8 days	16 days	32 days
Pullularia pullulans	2	8	25	26	49
Helminthosporium sp.	47	49	66	85	100
Alternaria solani	0	0	0	32	64
Memnoniella echinata	0	0	6	19	32
Penicillium citrinium	0	0	0	40	100
Aspergillus glaucus	0	47	100	100	100
Cladosporium fulvum	0	14	58	78	99
Rhizopus arrhizus	0	0	1	25	50
Monilia brunnea	2	9	19	41	66
Rhizopus stolonifer	2	9	79	100	100
Aspergillus fum <b>igatus</b>	0	0	0	10	17
Penicillium funiculosum	0	0	2	32	100
Aspergillus nidulans	0	0	7	15	42
Aspergillus niger	0	0	0	19	64
Trichoderma sp.	0	4	23	31	80
Myrothecium verrucaria	1	3	6	7	10
Aspergillus tamarii	0	o	3	14	43
Chaetomium globosum	0	0	3	7	14
Penicillium ochrochloron	0	0	0	4	32
Botrytis cinerea	0	9	0	36	95
Curvularia lunata	2	4	8	10	19

TABLE XXVIII

AVERAGED RESULTS OF SPORE GERMINATION TESTS

50°F and 85% R.H.

Organism	2 days	4 days	8 days	16 days	32 days
Pullularia pullulans	0	28	69	100	100
Helminthosporium sp.	0	0	39	40	47
Alternaria solani	0	0	0	0	0
Memnoniella echinata	0	0	0	0	8
Penicillium citrinium	0	0	0	0	1
Aspergillus glaucus	0	0	0	0	100
Cladosporium fulvum	0	0	1	2	100
Rhizopus arrhizus	0	0	0	0	15
Monilia brunnea	0	0	5	6	11
Rhizopus stolonifer	0	0	3	31	86
Aspergillus fumigatus	0	0	0	0	0
Penicillium funiculosum	0	1	6	10	15
Aspergillus nidulans	o	0	3	5	10
Aspergillus niger	0	0	0	0	0
Trichoderma sp.	0	1	8	10	29
Myrothecium verrucaria	0	0	0	1	2
Chaetomium globosum	0	0	0	0	0
Penicillium ochrochloron	0	0	0	0	0
Botrytis cinerea	0	0	0	0	2
Curvularia lunata	0	0	10	10	14

TABLE XXIX

AVERAGED RESULTS OF SPORE GERMINATION TESTS

50°F and 80% R.H.

Organism	2 days	4 days	8 days	16 days	32 days
Pullularia pullulans	0	2	20	54	100
Helminthosporium sp.	0	0	5	11	27
Alternaria solani	0	0	0	0	0
Memnoniella echinata	0	0	0	0	3
Penicillium citrinium	0	0	0	0	0
Aspergillus glaucus	0	0	0	0	95
Cladosporium fulvum	0	0	4	7	23
Rhizopus arrhizus	0	0	0	0	1
Monilia brunnea	0	0	3	3	6
Rhizopus stolonifer	0	0	3	17	46
Aspergillus fumigatus	0	0	0	0	0
Penicillium funiculosum	0	0	3	4	6
Aspergillus nidulans	0	0	2	6	13
Aspergillus niger	0	0	0	0	0
Trichoderma sp.	0	6	26	30	42
Myrothecium verrucaria	0	0	4	11	19
Aspergillus tamarii	0	0	3	3	4
Chaetomium globosum	0	0	3	4	9
Penicillium ochrochloron	0	0	0	0	0
Botrytis cinerea	0	0	0	0	1
Curvularia lunata	0	0	5	7	15

TABLE XXX 
AVERAGED RESULTS OF SPORE GERMINATION TESTS  $\underline{50}^{\circ}\text{F} \text{ and } 75\% \text{ R.H.}$ 

Organism	2 days	4 days	8 days	16 days	32 days
Pullularia pullulans	0	0	0	3	11
Helminthosporium sp.	0	0	0	0	0
Alternaria solani	0	0	0	0	0
Memnoniella echinata	0	0	0	0	0
Penicillium citrinium	0	0	0	0	0
Aspergillus glaucus	0	0	0	0	0
Cladosporium fulvum	0	0	0	0	0
Rhizopus arrhizus	0	0	0	0	0
Monilia brunnea	0	0	0	0	0
Rhizopus stolonifer	0	1	1	2	4
Aspergillus fumigatus	, <b>O</b>	0	0	0	0
Penicillium funiculosum	0	0	0	0	0
Aspergillus nidulans	0	0	0	0	0
Aspergillus niger	0	0	0	0	0
Trichoderma sp.	0	0	0	0	0
Myrothecium verrucaria	0	0	0	0	0
Aspergillus tamarii	0	0	0	0	0
Chaetomium globosum	0	0	0	0	0
Penicillium ochrochloron	o	0	o	0	2
Botrytis cinerea	0	0	0	0	0
Curvularia lunata	0	0	0	0	0

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Organism	2 days	4 days	8 days	16 days	32 days
Pullularia pullulans	o	0	0	0	7
Helminthosporium sp.	0	0	0	0	0
Alternaria solani	0	0	0	0	0
Memnoniella echinata	0	0	0	0	0
Penicillium citrinium	0	0	0	0	0
Aspergillus glaucus	0	0	0	0	0
Cladosporium fulvum	0	0	0	0	0
Rhizopus arrhizus	0	0	0	0	0
Monilia brunnea	0	0	0	0	0
Rhizopus stolonifer	0	0	0	1	3
Aspergillus fumigatus	0	0	0	0	0
Penicillium funiculosum	0	0	0	0	0
Aspergillus nidulans	0	0	0	0	0
Aspergillus niger	0	0	0	0	0
Trichoderma sp.	0	0	0	0	0
Myrothecium verrucaria	0	0	0	0	0
Aspergillus tamarii	0	0	0	0	0
Chaetomium globosum	0	0	0	0	0
Penicillium ochrochloron	0	0	0	0	1
Botrytis cinerea	o	0	0	0	0
Curvularia lunata	0	0	0	0	0

TABLE XXXII

AVERAGED RESULTS OF SPORE GERMINATION TESTS

50°F and 65% R.H.

Organism	2 days	4 days	8 days	16 days	32 days
Pullularia pullulans	0	0	0	0	0
Helminthosporium sp.	0	0	0	0	0
Alternaria solani	0	0	0	0	0
Memnoniella echinata	0	0	0	0	0
Penicillium citrinium	0	0	0	0	0
Aspergillus glaucus	0	0	0	0	0
Cladosporium fulvum	0	0	0	0	0
Rhizopus arrhizus	0	0	0	0	0
Monilia brunnea	0	0	0	0	0
Rhizopus stolonifer	0	0	0	0	2
Aspergillus fumigatus	0	0	0	0	0
Penicillium funiculosum	0	0	0	0	3
Aspergillus nidulans	0	0	0	0	0
Aspergillus niger	0	0	0	0	0
Trichoderma sp.	0	0	0	0	0
Myrothecium verrucaria	0	0	0	0	0
Aspergillus tamarii	0	0	0	0	1
Chaetomium globosum	0	0	0	0	0
Penicillium ochrochloron	0	0	0	0	0
Botrytis cinerea	0	0	0	0	0
Curvularia lunata	0	0	0	0	0

TABLE XXXIIT

AVERAGED RESULTS OF SPORE GERMINATION TESTS

### 50°F and 60% R.H.

Organism	2 days	4 days	8 days	16 days	32 days
Pullularia pullulans	0	0	0	1	2
Helminthosporium sp.	0	0	0	0	0
Alternaria solani	0	0	0	0	0
Memnoniella echinata	0	0	0	0	0
Penicillium citrinium	0	0	0	0	O
Aspergillus glaucus	0	0	0	0:	0
Cladosporium fulvum	0	0	0	0	0
Rhizopus arrhizus	0	0	0	0	0
Monilia brunnea	0	0	0	0	0
Rhizopus stolonifer	0	0	0	0	1
Aspergillus fumigatus	0	0	0	0	0
Penicillium funiculosum	0	0	0	0	0
Aspergillus nidulans	0	0	0	0	0
Aspergillus niger	0	0	0	0	0
Trichoderma sp.	0	0	0	0	0
Myrothecium verrucaria	0	0	0	0	0
Aspergillus tamarii	0	0	0	0	0
Chaetomium globosum	0	0	0	0	0
Penicillium ochrochloron	0	0	0	0	0
Botrytis cinerea	0	0	0	0	0
Curvularia lunata	0	o	0	0	0

TABLE XXXIV

AVERAGED DATA FOR EXAMINATION OF THE EFFECT OF TEMPERATURE ON GERMINATION\*

Temperature		Time in Days				
(°F)	<u>2</u>	<u>4</u>	<u>8</u>	<u>16</u>	<u>32</u>	
100	2.51	9.35	15.5	24.2	30.1	
80	2.29	7.38	14.8	22.6	27.0	
70	1.01	2.61	5.70	12.6	22.8	
60	0.26	1.45	4.52	11.9	15.9	
50	0.80	1.96	6.94	11.2	19.9	

<sup>\*</sup> Each figure represents the average of the germination percentages for all organisms at all relative humidities at each temperature.

TABLE XXXV

AVERAGED DATA FOR THE EXAMINATION OF THE EFFECT OF RELATIVE HUMIDITY ON GERMINATION\*

Relative Humidity		Time in Days				
<u>(%)</u>	<u>2</u>	<u>4</u>	<u>8</u>	<u>16</u>	<u>32</u>	
95	8.88	27.1	50.5	70.7	86.4	
90	4.03	13.7	29.4	56.8	75.9	
85	0.56	2.95	9.48	25.7	44.3	
80	0.28	1.65	5.26	11.4	23.3	
75	0.01	0.08	0.14	0.28	0.86	
70	0.00	0.08	0.11	0.22	0.63	
65	0.00	0.00	0.00	0.00	0.06	
60	0.00	0.00	0.00	0.01	0.03	

<sup>\*</sup>Each figure represents the average of the germination percentages for all organisms at all temperatures at each relative humidity.

I. Block, S.S. II. Rodriguez-Torrent, R. III. Cole, M.B. IV. Aeronautical Systems Division, Aerospace Medical Laboratory, Wright-Patterson Air Force Base, Ohio. V. Contract AF33(616)-6387	UNCLASSIFIED
ASD TR 61-490 University of Florida, Gainesville, Florida HUMIDITY AND TEMPERATURE REQUIRE-MENTS OF SELECTED FUNGI, by Seymour S. Block, Ralph Rodriguez-Torrent, and Margaret B. Cole. October 1961. 56 pp., incl. illus., and tables. (Proj. 7312, 7165; Task 71837) (Contr. No. AF33(616)-6387)Unclassified report the viability of spores of selected funge at various combinations of relative humidity between 60 and 95 percent and temperatures between 50° and 100°F. The microorganisms were selected because they were known to be a cause of, or associated with, fungal	deterioration of Air Force materiel. Spore germination was usually prevented when the relative humidity was below 70 percent regardless of temperature. Reducing the temperature at R. H. values below 85 percent may retard spore germination and growth slightly. Above 90 percent R. H., any reduction in temperature has very little effect on spore germination.
I. Block, S.S. II. Rodriguez-Torrent, R. III. Cole, M.B. IV. Aeronautical Systems Division, Aerospace Medical Laboratory, Wright-Patterson Air Force Base, Ohio. V. Contract AF33(616)- 6387	UNCLASSIFIED
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